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INDIVIDUAL AND COMBINED EFFECTS OF SALINITY AND TEMPERATURE ON EMBRYOS AND LARVAE OF THE COOT CLAM, *MULINIA LATERALIS* (SAY) [1]

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Thorson's 1946 publication on the early life history of Danish marine bottom invertebrates has helped establish the field of marine benthic and larval ecology. A notable lack of knowledge still exists, nevertheless, regarding the life history and autecology of the majority of organisms that are part of bottom communities.

Until recently the rearing of marine bivalves was virtually impossible because of the lack of reliable methods. The relatively new development of techniques for rearing marine bivalves (Loosanoff and Davis, 1963) has provided further incentive, however, for autecological studies of larvae. By the successful conditioning and spawning of adult bivalves the larvae of many species have been reared under controlled conditions (Loosanoff and Davis, 1963; Stickney, 1964; Walne, 1964, 1966; Bayne, 1965; Chanley, 1965a, 1965b, 1966, 1967; Chanley and Castagna, 1966; Loosanoff, Davis and Chanley, 1966). These techniques make it possible to subject bivalve embryos and larvae to various ecological factors under controlled experimental conditions.

Little is known about the combined effect of two or more environmental factors on marine animals. Medcof and Needler (1941) attempted to deduce the interaction of temperature and salinity on the condition index of the American oyster, *Crassostrea virginica*, in natural waters. Costlow, Bookhout, and Monroe (1960, 1962) studied the combined effects of temperature and salinity on development of eggs and larvae of the decapod crustaceans, *Sesarma cinereum* and *Panopeus herbstii*. Kinne (1963, p. 302) reviewed the existing knowledge of the effects of temperature and salinity on marine and brackish water fish and emphasized the fact that "monofactorial analysis may lead to conclusions that are ecologically invalid," and "should be replaced wherever possible by bi, tri, or polyfactorial approach." This view influenced Davis and Calabrese (1964) to study the effects of variations in such factors as food and salinity on the temperature

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tolerance of embryos and larvae of the American oyster, *C. virginica,* and the hard shell clam, *Mercenaria mercenaria.*

The present study of the individual and combined effects of salinity and temperature on embryos and larvae of *Mulinia lateralis* (Say) shows the interaction of these factors in the laboratory; the tolerances established, however, may be further modified in nature where other environmental factors may also affect the temperature and salinity tolerances of these clams.

Methods

Methods for maintaining bivalves in spawning condition and obtaining fertilized eggs throughout the year were described by Loosanoff and Davis (1963). To determine the effect of salinity and temperature, individually and in combination, on embryonic development of *M. lateralis,* 12,000 to 15,000 fertilized eggs, from parents that were held at the same initial temperature and salinity, were placed into each of a series of 1-liter, polypropylene beakers. To determine the effect of the different factors on embryonic development, the larvae from each culture were collected, after 48 hours at the experimental conditions, on a stainless steel screen of mesh size small enough to retain them. The larvae were resuspended in a 250-ml graduated cylinder and, after thorough stirring to insure uniform distribution of the larvae, a 4-ml quantitative sample was removed and preserved in 5% neutral formalin. The samples were examined under a compound microscope (× 125) and the number of larvae that had developed normally was counted. To compare the number of embryos developing to normal straight-hinge larvae in successive experiments, the results were calculated as the percentage of the maximum number developing normally at any salinity and temperature (either individually or in combination) in that experiment.

To ascertain the effect of the various test conditions on survival and growth, I placed 10,000 to 14,000 larvae, which had been reared to the 48-hour straight-hinge stage under normal conditions (27 ± 0.5‰ salinity and 25 ± 1° C), into each of the series of 1-liter beakers. The sea water in all cultures was changed 3 times a week to eliminate metabolic waste products, and experimental conditions were re-established. In all experiments 50 mg/l of Sulmet were routinely added to all cultures to prevent possible disease-induced mortality that was not a direct effect of the factor being tested. (Sulmet, sodium sulfamethazine, is a trade name of American Cyanamid Co. Mention of trade names does not imply endorsement of the product by the Bureau of Commercial Fisheries.) In experiments involving only temperature, supplemental algal food, consisting of a mixture of *Isochrysis galbana, Monochrysis lutheri,* and *Chlorella* sp. 580 (Indiana University Collection #580), was added daily by the procedures of Davis and Guillard (1958). To keep salinities constant in tests involving salinity, however, food was added only when the water was changed and salinities were adjusted. Experiments were discontinued when larvae in the fastest growing cultures reached setting size (6 to 8 days). When the experiments were terminated, quantitative samples were taken as in the experiments on embryonic development. The number of larvae that had survived the experimental treatment was counted and 100 (if available) were measured to the nearest 5 μ with an ocular micrometer in a compound microscope. The number of larvae that survived and the increase in mean length in each experi-

ment were calculated as percentages of the maximum number that survived and the increase in length of larvae in the most rapidly growing cultures, respectively, at any salinity and temperature (either individually or in combination) in that experiment. The method for determining the number of larvae surviving or the percentage of bivalve embryos developing into normal straight-hinge larvae is considered accurate to about ± 10% (Davis, 1958).

Four experiments were conducted at salinities of 7.5 to 37.5‰ at 2.5‰ intervals. The effect of each salinity was tested in at least 2 experiments and some salinities were repeated in all 4 experiments. All cultures were kept in a constant temperature bath at 25 ± 1° C. Salinities below 27‰ were prepared by diluting laboratory sea water with demineralized tap water, and salinities above 27‰ were made up by adding sea water, concentrated by evaporation, to the laboratory sea water. All salinities were determined by the hydrometer method and the use of Knudsen's Tables (1901). Eight experiments were conducted at temperatures of 7.5 to 34.5° C at 2.5° C intervals. The effect of each temperature was tested in at least 2 experiments and some temperatures were repeated in as many as 5 experiments. Duplicate cultures were established at each test condition. The salinity of the sea water in these experiments was 27 ± 0.5‰.

Three experiments were conducted with salinities of 10 to 35‰ at 5‰ intervals in combination with temperatures from 7.5 to 32.5° C at 5° C intervals. Duplicate cultures were established at each of the 6 salinities and 6 temperatures tested, giving a total of 36 combinations and 72 cultures in each experiment. Low salinity sea water was made up by dilution with demineralized tap water. I obtained highly concentrated sea water by first freezing some laboratory sea water and then later melting off just a small portion. This highly concentrated sea water was then used to make up the sea water for high salinity studies.

TABLE I

Percentage of embryos developing, larvae surviving, and increase in mean length of larvae of M. lateralis *at different salinities*

Salinity* (o/oo)	Percentage development of embryos	Percentage survival of larvae	Percentage increase in mean length of larvae
7.5	0.0	5.8	6.0
10.0	0.0	19.1	19.4
12.5	0.0	35.0	36.7
15.0	10.1	32.6	42.4
17.5	15.6	58.6	61.9
20.0	59.8	87.7	78.7
22.5	83.5	83.4	78.9
25.0	91.4	93.7	100.0
27.5	97.0	94.6	82.4
30.0	81.1	61.4	75.1
32.5	61.9	64.4	68.8
35.0	14.9	38.5	54.0
37.5	1.2	40.7	61.7

* All cultures were maintained at 25 ± 1° C.

Results

Effect of salinity

M. lateralis embryos developed into normal straight-hinge larvae throughout the relatively wide salinity range from 15 to 35‰ at 25 ± 1° C (Table I and Fig. 1). At a salinity of 37.5‰ only 1.2% of the embryos developed into normal shelled

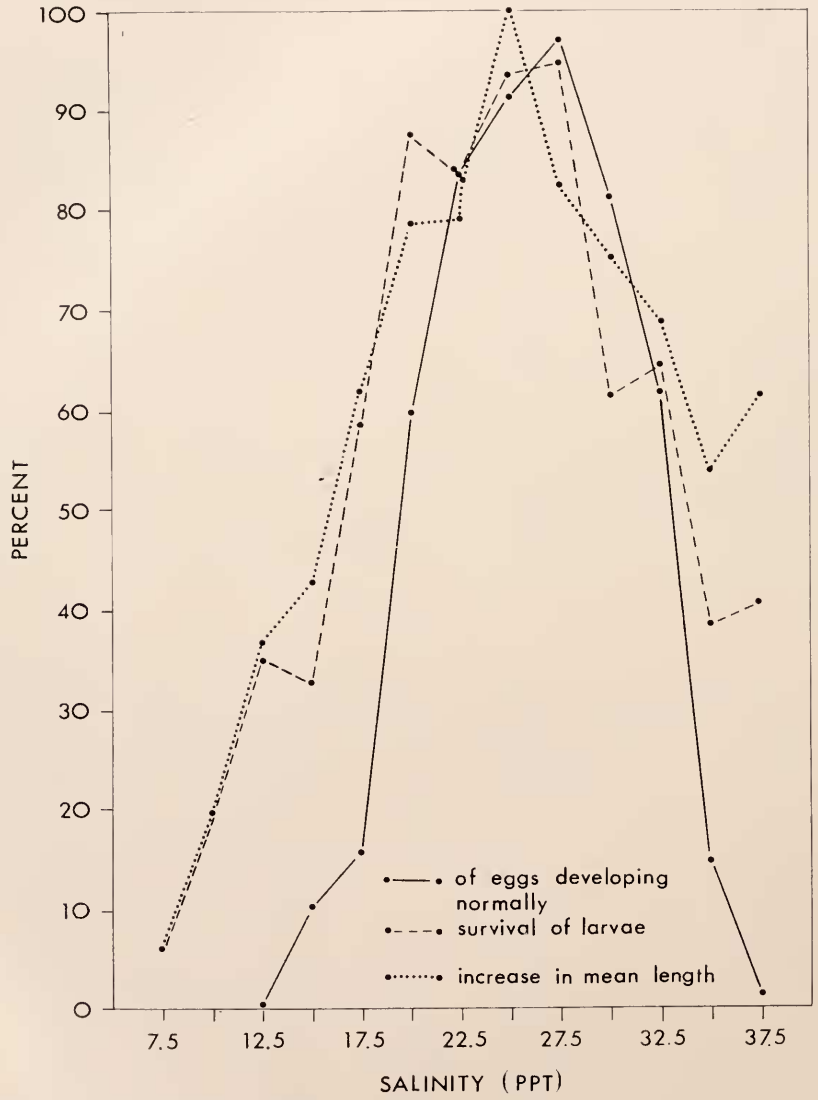

FIGURE 1. The salinity tolerance of embryos and larvae of *M. lateralis* at 25 ± 1° C, as indicated by percentage of embryos that developed normally, percentage of larvae that survived, and percentage increase in mean length of larvae.

TABLE II

Percentage of embryos developing, larvae surviving, and increase in mean length of larvae of M. lateralis *at different temperatures*

Temperature* (°C)	Percentage development of embryos	Percentage survival of larvae	Percentage increase in mean length of larvae
7.5	0.0	86.1	3.7
10.0	17.3	81.0	5.7
12.5	60.9	89.7	14.2
15.0	75.1	92.7	25.3
17.5	92.8	85.8	41.9
20.0	98.9	93.5	69.5
22.5	92.4	92.0	75.6
25.0	92.1	90.4	79.2
27.5	43.3	88.4	93.5
30.0	39.0	60.1	79.4
32.5	0.0	29.9	64.2
34.5	—	0.0	0.0

* All cultures were maintained at 27 ± 0.5‰ salinity.

larvae, and at 35‰ 14.9% developed normally. At 15 and 17.5‰ 10.1 and 15.6%, respectively, of the embryos developed normally, and at 12.5‰ none developed. Approximately 60% of the embryos developed into shelled larvae at 20 and 32.5‰, but the salinity range for a satisfactory percentage of fertilized eggs (70% or more of maximum) to develop was from 22.5 to 30‰. With temperature constant at 25 ± 1° C the optimum salinity for embryonic development, *i.e.*, the salinity at which the highest percentage of straight-hinge larvae was obtained, was 25 to 27.5‰. Thus, the salinity range from 22.5 to 30‰ for satisfactory development of embryos into normal shelled larvae approximates the salinity range of 22.5 to 30‰ for satisfactory development of hard shell clam embryos, as reported by Davis (1958).

Once larvae reached the straight-hinge stage, some were able to survive at salinities from 7.5 to 37.5‰ (Table I and Fig. 1), a range much wider than that tolerated by developing embryos. Only a small percentage (5.8) of larvae, however, survived at 7.5‰, and only 32.6% at 15‰. The upper salinity limit for larval survival was not determined since at 37.5‰, the highest salinity tested, a rather high percentage (40.7) of larvae survived. A satisfactory percentage (70% or more of maximum) survived at salinities from 20 to 27.5‰, and optimum survival was at 25 to 27.5‰. Thus, the optimum salinity for both developing embryos and survival of larvae was 25 to 27.5‰.

Larvae were able to grow satisfactorily (70% or more of maximum) within the salinity range from 20 to 30 or 32.5‰; the optimum growth was at 25‰ (Table I and Fig. 1). The range for satisfactory growth was, therefore, slightly greater than that for satisfactory survival. Growth was most limited at 7.5 and 10‰ (less than 20% of maximum), but the rate of growth increased gradually as the salinity was increased from 12.5‰ (36.7%) to 17.5‰ (61.9%). The rate of growth decreased slowly at salinities above 27.5‰ but even at 37.5‰ (the highest salinity tested) the increase in mean length of larvae (61.7%) was substantial.

Effect of temperature

At a constant salinity of 27 ± 0.5‰ *M. lateralis* embryos did not develop at 7.5° C, but at 10° C a small percentage (17.3) developed into straight-hinge larvae (Table II and Fig. 2). The percentage of fertilized eggs developing normally increased sharply from 17.3 at 10° C to 60.9 and 75.1 at 12.5 and 15° C, respectively. Differences in the percentage of straight-hinge larvae obtained at temperatures of 17.5, 20, 22.5, and 25° C were not significant, although the data indicate that 20° C may be near the optimum. The percentage of embryos that developed normally was greatly reduced at 27.5 and 30° C (43.3 and 39, respectively) and none developed at 32.5° C.

Although no embryos developed at 7.5° C, it was evident that larvae could tolerate 7.5° C at least for 10 days with little mortality (Table II and Fig. 2). A total of 86.1% of the larvae survived at 7.5° C, but growth was negligible. Even though the larvae were capable of surviving this cold temperature for the duration of the laboratory experiments, growth was so slow as to make it obvious that all would have eventually died before metamorphosis. More than 80% of the larvae survived at temperatures from 7.5 to 27.5° C, but the percentage dropped sharply to 60.1 at 30° C, and no larvae survived at 34.5° C.

The average rate of growth of larvae increased progressively with each increase in temperature from 7.5 to 27.5° C and then progressively decreased at temperatures of 30 and 32.5° C (Table II and Fig. 2). Growth was satisfactory only within the range from 20 to 30° C. Larvae grew most rapidly at 27.5° C, the temperature found optimum for growth of larvae of the European oyster, *Ostrea edulis* (Davis and Calabrese, 1969).

Combined effects of salinity and temperature

The temperature and salinity requirements for development of *M. lateralis* embryos to straight-hinge larvae are shown in Table III. From 83.3 to 95.9% of the embryos developed into straight-hinge larvae within the dash-lined boundary which was circumscribed by temperatures from 12.5 to 27.5° C and salinities from

TABLE III

Percentage development of M. lateralis *embryos to straight-hinge larvae at different combinations of salinity and temperature*

Salinity (o/oo)	Temperature (°C)					
	32.5	27.5*	22.5	17.5	12.5*	7.5
35.0	1.0	41.3	58.5	42.8	2.6	0.0
30.0	20.3	88.5	93.9	89.1	83.4	0.0
25.0	31.6	84.8	95.9	91.8	69.2	0.0
20.0	27.3	84.5	91.4	83.3	41.7	0.0
15.0	1.5	6.4	12.8	0.7	0.0	0.0
10.0	0.0	0.0	0.0	0.0	0.0	0.0

* Results of two experiments—temperature control unit went out of order in third experiment.

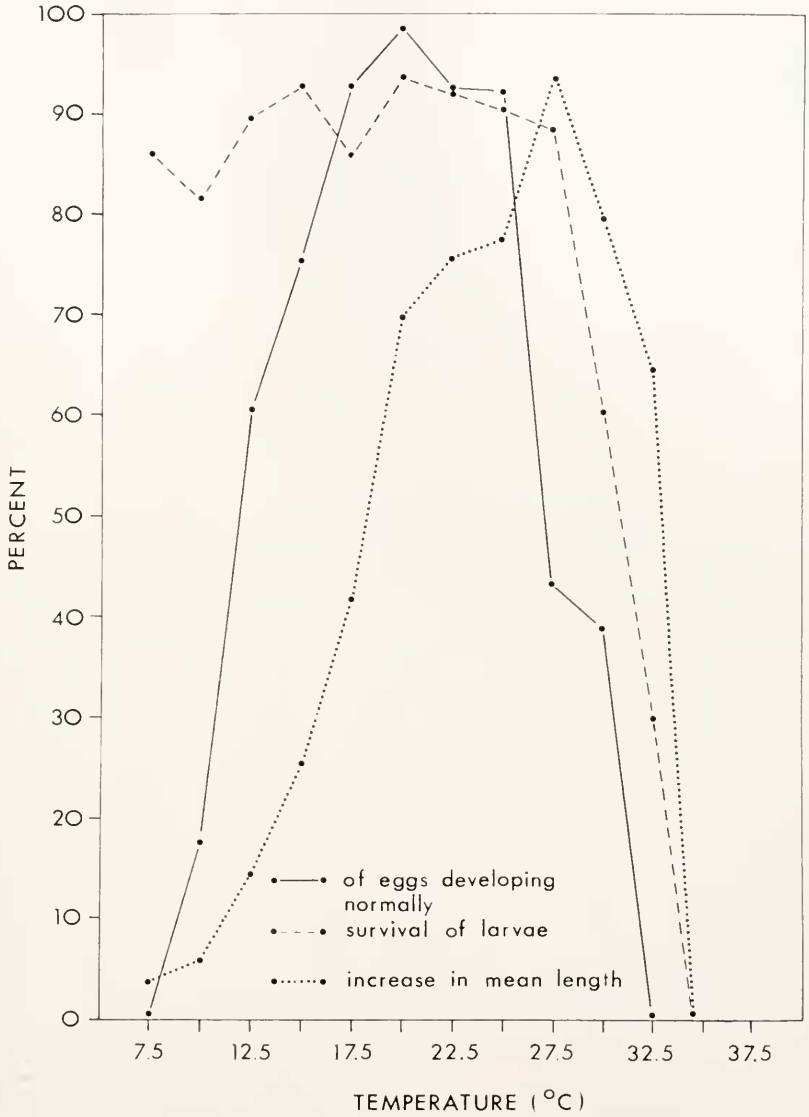

FIGURE 2. The temperature tolerance of embryos and larvae of *M. lateralis* at 27 ± 0.5‰ salinity, as indicated by the percentage of embryos that developed normally, percentage of larvae that survived, and percentage increase in mean length of larvae.

20 to 30‰. At 32.5° C the percentage of embryos that developed normally was drastically reduced at all salinities and at 7.5° C no normal larvae developed at any salinity. The temperature of 12.5° C appears to be "borderline" in that a normal number of embryos developed into straight-hinge larvae at 30‰ salinity, but 69.2% developed at 25‰ (this percentage is almost precisely at the 70% level

TABLE IV

Percentage survival of M. lateralis *larvae at different combinations of salinity and temperature*

Salinity (o oo)	Temperature (°C)					
	32.5	27.5	22.5	17.5	12.5	7.5
35.0	32.1	76.2	83.4	92.6	84.7	75.1
30.0	75.1	82.4	92.6	89.8	84.3	75.2
25.0	32.2	77.9	90.8	88.2	81.6	80.7
20.0	58.7	82.7	82.9	85.8	83.1	82.3
15.0	25.4	75.9	85.6	79.3	80.7	75.9
10.0	2.2	63.9	77.7	77.9	66.5	62.4

previously discussed as of ecological significance), and 41.7% of the embryos developed normally at 20‰. At 35‰ salinity the percentage of embryos developing normally depended upon temperature, but at none of the temperatures tested did as many as 70% develop into normal straight-hinge larvae. A maximum of 58.5% developed normally at 22.5° C and 35‰ salinity. This salinity was obviously above optimum and approached the upper limit for embryonic development of *M. lateralis*. Only a few normal larvae were obtained at 15‰ salinity; the highest percentage (12.8) was at 22.5° C. No normal larvae developed at 10‰ at any temperature. For a satisfactory percentage of embryos to develop into straight-hinge larvae the salinity must remain between 20 and 30‰ and the temperature from 12.5 to 27.5° C (or 17.5° C if salinity is below 25‰).

Once the embryos developed to straight-hinge larvae, more than 70% survived at almost all combinations of temperature and salinity tested (Table IV). Erratic survival at 32.5° C, however, indicates that this temperature approached the upper limit for survival; only at a salinity of 30‰ was survival at this temperature above 70%. At low temperatures (12.5 and 7.5° C) survival of larvae was satisfactory at all salinities except 10‰, although growth was almost nil (Table V). At 10‰

TABLE V

Percentage increase in mean length of M. lateralis *larvae at different combinations of salinity and temperature*

Salinity (o /oo)	Temperature (°C)					
	32.5	27.5	22.5	17.5	12.5	7.5
35.0	63.5	83.6	70.2	25.4	2.7	1.2
30.0	65.0	99.4	90.5	43.1	3.2	0.7
25.0	45.6	81.0	82.3	43.2	4.9	0.7
20.0	61.7	84.6	92.9	61.7	13.3	1.1
15.0	38.8	57.5	68.2	38.6	3.5	1.2
10.0	0.0	40.9	44.9	15.8	0.9	0.5

salinity, even though survival was about normal at temperatures of 17.5 and 22.5° C and fair at 7.5, 12.5, and 27.5° C, growth was poor.

It is obvious from Tables III, IV, and V that larvae can survive throughout a wider range of temperature and salinity than is satisfactory for either embryonic development or growth of larvae. In these experiments growth of larvae was satisfactory only at temperatures from 22.5 to 27.5° C and salinities from 20 to 35‰. Temperatures of 17.5° C and lower were obviously below optimum, and 32.5° C was above optimum for growth. Growth, like survival, was erratic at 32.5° C. This temperature favors bacterial growth; probably the effects on survival and growth of larvae were the combined results of the increased bacteria and bacterial toxins and the reduced resistance of the larvae.

Satisfactory areas for reproduction of *M. lateralis* would appear to be limited to those where salinities are 20‰ or higher, as determined by the limits for embryonic development and growth, and where temperatures are approximately 22.5 to 27.5° C, as determined by the limits for satisfactory growth.

Discussion

Temperature has the greatest influence on the duration of the pelagic stage. Low water temperatures prolong pelagic life and high temperatures shorten it (provided food is adequate). Temperatures during the actual spawning season of *M. lateralis* in Long Island Sound never surpass the high limits that are lethal to developing eggs and larvae. Thorson (1950) suggested that the area of distribution of a species may comprise several sub areas in relation to temperature: one in which the temperature range permits the adult animals to grow, a somewhat more restricted area in which it permits development of gametes and spawning as well as growth (spawning appears to require a somewhat higher temperature than the process of ripening), and an even smaller area in which embryos and larvae successfully develop. Thorson also stated that temperatures required for spawning are so high and definitely limited that they will normally allow fertilized eggs and larvae to develop regularly, provided that other conditions are favorable; the same holds true for *M. lateralis*. Adults have survived temperatures at least as low as −2° C in outdoor tanks at the Milford laboratory and at the highest temperatures normally found in Long Island Sound (about 24° C). Gametogenic activity occurred at temperatures from about −0.1° C to normal spawning temperatures of about 20° C. From laboratory experiments it was determined that eggs developed from 10 to 30° C, and larvae survived at temperatures from 7.5 (the lowest tested) to 32.5° C.

Davis and Calabrese (1964) suggested that the failure of bivalve larvae to grow at low temperatures appeared to be caused by their inability to digest available food. Their experiments demonstrated that larvae of the American oyster and hard shell clam survived for long periods and ingested food at temperatures below the minimum at which they grew. They also suggested that enzymes required to digest naked flagellates, such as *I. galbana* and *M. luthcri*, were perhaps active at lower temperatures than those involved in the digestion of certain other forms with thick cell walls, such as *Chlorella*. Davis and Calabrese (1964) stated that the increase in growth rate of larvae at higher temperatures probably resulted from increased activity of the enzyme system at the higher temperatures. Ukeles (1961)

demonstrated that temperatures of 27° C or higher destroyed the cells of *I. galbana* and *M. lutheri*, although cells of *Chlorella* sp. 580 survived even at 33° C. The reduction in growth of *M. lateralis* larvae at 32.5° C, then, may have represented partly an indirect effect of temperature on the food organisms. Since reduction of larval survival was also drastic at this temperature, it can be concluded that temperature also affected survival directly and, perhaps, growth. Davis and Calabrese (1969) studied the effect of temperature on European oyster larvae using the same 3 species of algal foods mentioned above. They believed that these foods were probably adequate at both temperature extremes, but did not preclude the possibility that other species of algae might have provided better growth at even lower or higher temperatures.

Salinity is a relatively stable factor in Long Island Sound; as with temperature, however, the area of distribution of an organism may comprise one in which the salinity range permits the adult animals to grow, possibly a smaller area in which the salinity permits development of gametes and spawning as well as growth, and an even more restricted area in which successful development of embryos and larvae is possible. Breuer (1957), in his studies of Alazan Bay, Texas, found *M. lateralis* in areas with salinities ranging from 1.4 to 75.1‰ and averaging 50.7‰. This extremely wide range does not necessarily mean that *M. lateralis* from all geographical areas are able to withstand such extreme salinities, nor does it mean that all stages of reproduction are accomplished at the extremes of the salinity range. In my studies I determined that embryos of *M. lateralis* from Long Island Sound did not develop below 15‰, and that at 37.5‰ (the highest salinity tested) only a negligible percentage developed. For an adult population to be established in areas of extreme salinity, one of two conditions would be necessary: (1) the salinity in that area would have to be within the range for embryonic development during the spawning season, or (2) spawning and embryonic development could take place in areas of suitable salinity and the larvae carried to areas of extreme salinity through dispersal. Additional research is needed to determine the minimum salinity at which *M. lateralis* develop gonads and whether the salinity at which the parent stock develops gonads influences the salinity tolerance of embryos and larvae.

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Summary

1. Embryos of *M. lateralis* held at 25 ± 1° C developed satisfactorily (70% or more of maximum) within the salinity range from 22.5 to 30‰; 27.5‰ was optimum. Some embryos developed normally, however, at salinities as low as 15‰ (10%) and as high as 37.5‰ (1.2%).

2. Some larvae survived at all salinities tested (7.5 to 37.5‰); survival was 70% or more only within the range from 20 to 27.5‰.

3. Larvae grew satisfactorily within the salinity range from 20 to 30 or 32.5‰; 25‰ was optimum.

4. Embryos held at 27 ± 0.5‰ salinity developed satisfactorily within the temperature range from 15 to 25° C; 20° C was optimum. Some embryos developed normally, however, at temperatures as low as 10 (17.3‰) and as high as 30° C (39‰).

5. Some larvae survived at temperatures from 7.5 (lowest tested) to 32.5° C; survival was satisfactory from 7.5 to 27.5° C.

6. Larvae grew satisfactorily at temperatures from 20 to 30° C; 27.5° C was optimum.

7. The effects of salinity and temperature were significantly related only when the tolerance of either one or the other was approached. When the salinity was unfavorable, the range of temperature was markedly narrowed and, conversely, when both salinity and temperature were within the satisfactory range there was no significant interrelationship.

8. The range of temperature tolerance for embryos narrowed above and below 30‰ salinity, and salinity tolerance narrowed above and below 22.5° C.

9. Survival of larvae was relative uniform at temperatures of 7.5 to 27.5° C and at salinities from 10 to 35‰, but at 32.5° C (at all salinities other than 30‰) the percentage of larvae surviving was drastically reduced.

10. Growth of larvae was most rapid within the salinity range from 20 to 35‰ and within the temperature range from 22.5 to 27.5° C.

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